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基础研究

GST与FGF23 C末端 71个氨基酸融合蛋白的 表达、纯化及其免疫原性

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摘要:

目的:构建和表达谷胱甘肽-S-转移酶（GST）与成纤维细胞生长因子23(FGF-23)的活性片段-C末端71个氨基酸（FGF23 CTR）融合蛋白的基因工程菌，并检测融合蛋白的免疫原性，为制备FGF23特异性单克隆抗体以及研发慢性肾病诊断试剂提供实验数据。方法：以质粒pET22b-fgf23为模板利用PCR方法扩增FGF23 CTR的基因片段，将该基因与表达载体pGEX-4T-1连接后转化至BL21宿主细胞中，通过IPTG诱导获得可溶性表达，将GST-FGF23 CTR融合蛋白用Glutathione Sepharose4B亲和层析法纯化，Western blotting法鉴定蛋白。该融合蛋白免疫Balb/C小鼠制备抗血清，采用ELISA法检测抗血清的效价。结果：构建GST-FGF23 CTR融合蛋白的表达载体，纯化后得到纯度90%以上的融合蛋白，并与商品化的FGF23多克隆抗体呈阳性反应，ELISA法证实融合蛋白具有良好的免疫原性。结论：成功构建GST-FGF23 CTR融合蛋白基因工程菌，并纯化得到具有良好免疫原性的融合蛋白，以此方法制备的抗原可用于FGF23特异性单克隆抗体的制备和FGF23生物学功能研究。

关键词: 成纤维细胞生长因子23；免疫原性；基因工程菌

Expression and purification of GST and FGF23 C termination fusion protein and its immunogenicity

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Abstract:

To construct the genetic engineering bacteria highly expressing fusion protein of glutathione-S-transferase (GST) and C-terminal 71 amino acids of fibroblast growth factor 23 (FGF23 CTR),and to test the immunogenicity of the fusion protein and to provide experimental data for FGF23 specific monoclonal antibodies and the development of diagnosis reagent for chronic kidney disease.Methods After FGF23 CTR gene fragment was obtained by PCR,it was fused with GST by expression vector pGEX-4T-1,then was transformed in E.coli BL21(DE3). By inducing with 1IPTG,the fusion proteins were expressed solubly.The fused protein was purified by Glutathione Sepharose4B.The purity of GST-FGF23 CTR by SDS-PAGE was shown to be higher than 90%.The Balb/C mice were immuned with fusion protein to pepare antiserum,then ELISA was used to assay antiserum titer.Results The GST-FGF23 CTR expression vector was constructed successfully.The purity of fusion protein was more than 90% after purification,and it positively reacted to the commercialization of FGF23 polyclonal antibody.The ELISA result showed that the confirmed fusion protein had good immunogenicity.Conclusion The genetic engineering bacteria of GST-FGF23 CTR is successfully constructed and the purified protein has good immunogenicity.The fused protein can be used for the FGF23 monoclonal antibody preparation and research on the biological function of FGF23.

Keywords: fibroblast growth factor 23;immunogenicity;genetic engineering bacteria

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